

BRIEF COMMUNICATION

Kidney allograft biopsy findings after COVID-19

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COVID-19 has been associated with acute kidney injury and published reports of native kidney biopsies have reported diverse pathologies. Case series directed specifically to kidney allograft biopsy findings in the setting of COVID-19 are lacking. We evaluated 18 kidney transplant recipients who were infected with SARS-CoV-2 and underwent allograft biopsy. Patients had a median age of 55 years, six were female, and five were Black. Fifteen patients developed COVID-19 pneumonia, of which five required mechanical ventilation. Notably, five of 11 (45%) biopsies obtained within 1 month of positive SARS-CoV-2 PCR showed acute rejection (four with arteritis, three of which were not associated with reduced immunosuppression). The remaining six biopsies revealed podocytopathy ($n = 2$, collapsing glomerulopathy and lupus podocytopathy), acute tubular injury ($n = 2$), infarction ($n = 1$), and transplant glomerulopathy ($n = 1$). Biopsies performed >1 month after positive SARS-CoV-2 PCR revealed collapsing glomerulopathy ($n = 1$), acute tubular injury ($n = 1$), and nonspecific histologic findings ($n = 5$). No direct viral infection of the kidney allograft was detected by immunohistochemistry, *in situ* hybridization, or electron microscopy. On follow-up, two patients died and most patients showed persistent allograft dysfunction. In conclusion, we demonstrate diverse causes of kidney allograft dysfunction after COVID-19, the most common being acute rejection with arteritis.

KEYWORDS

biopsy, clinical research / practice, complication: infectious, infection and infectious agents - viral, kidney (allograft) function / dysfunction, kidney transplantation / nephrology

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) has been associated with acute kidney injury (AKI). Whereas a growing body of literature

describes the pathologic findings associated with COVID-19 in native kidney biopsies,¹⁻⁵ there is a paucity of information about COVID-19-associated pathology in kidney transplant patients. To our knowledge, only 15 kidney allograft biopsy findings from

Abbreviations: ACE2, angiotensin-converting enzyme 2; AKI, acute kidney injury; AMR, antibody-mediated rejection; APOL1, apolipoprotein L1; ATI, acute tubular injury; CNI, calcineurin inhibitor; COVID-19, coronavirus disease 2019; CUIMC, Columbia University Irving Medical Center; DSAs, donor-specific antibodies; FFPE, formalin-fixed paraffin-embedded; IQR, interquartile range; IRB, Institutional Review Board; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCMR, T cell-mediated rejection; TMA, thrombotic microangiopathy.

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COVID-19-infected transplant recipients have been published as case reports ($n = 9$) or as part of series containing predominantly native kidney biopsies ($n = 6$).^{1,6-15} These reports (summarized in Table S1) demonstrate a variety of etiologies of allograft dysfunction in the transplanted kidney, including podocytopathies,^{6,9-13} acute rejection,^{1,15} allograft infarction,¹⁴ thrombotic microangiopathy (TMA),⁷ and acute tubular injury (ATI).^{1,8,15} Direct viral invasion of kidney parenchyma was suggested in two reported cases.^{8,9} Because individual case reports may be subject to publication bias and the timeline relative to COVID-19 infection was not provided consistently, detailed case series devoted to kidney allograft biopsy findings are needed to elucidate the range of kidney manifestations and inform clinical management. Herein, we report the first case series of kidney allograft biopsy findings from recipients with COVID-19.

2 | METHODS

From the archives of the Renal Pathology Laboratory at Columbia University Irving Medical Center (CUIMC), we retrospectively identified kidney allograft biopsies from SARS-CoV-2-infected patients procured between 3/2020 and 5/2021. All biopsies were “for-cause” biopsies, largely reflecting elimination of protocol biopsies during the pandemic. Patients were required to have either a positive SARS-CoV-2 PCR by nasal swab within 60 days of biopsy or more than 60 days before biopsy plus no documentation of a subsequently negative PCR test result performed prior to the allograft biopsy ($n = 18$). We chose this arbitrary period because transplant patients are known to have a prolonged COVID-19 course^{16,17} and the potential duration of COVID-19-related histologic manifestations is unknown.

To be more precise regarding the effects of COVID-19, we divided patients into those who had recent infection (positive PCR ≤ 1 month of biopsy, $n = 11$), and those with more “remote” infection (positive PCR > 1 month of biopsy, $n = 7$). These allograft biopsies were reviewed at CUIMC and included 17 biopsies processed at CUIMC and one biopsy referred to CUIMC for immunostaining for SARS-CoV-2. Three of these biopsies were previously reported by our group.¹ Clinical and laboratory data were extracted from chart review or provided by the referring physician.

All biopsies were processed for light microscopy and immunofluorescence staining for C4d. A full immunofluorescence panel (IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrinogen, albumin) was performed on all but three patients with minimal proteinuria (< 0.2 g/g). Electron microscopic evaluation was performed for six patients. All formalin-fixed paraffin-embedded (FFPE) biopsies underwent immunohistochemical staining for the SARS-CoV-2 nucleocapsid protein (SARS-N-Capsid) using rabbit monoclonal antibody from clone 001 (catalog no. 40143-R001; Sino Biologic, Beijing, People's Republic of China), and 17 FFPE biopsies underwent *in situ* hybridization for SARS-CoV-2 RNA-encoding spike protein (RNA scope 2.5 LS Probe V-CoV2019-S, catalog no. 848568; Advanced Cell Diagnostics).

In an attempt to identify histologic correlates of COVID-19 in the kidney allograft, we compared biopsy findings in our cohort with recent COVID-19 to these of 14 kidney transplant recipients who had non-COVID pneumonia within 1 month before allograft tissue collection (13 autopsies and 1 “for-cause” biopsy), 13 native kidney biopsies obtained within 1 month of COVID, and all pre-COVID “for-cause” kidney allograft biopsies (protocol biopsies were excluded) reviewed at CUIMC between January 1, 2019 and December 31, 2019 ($n = 538$). Pneumonia was defined by an infectious disease specialist (MRP) based on a combination of clinical symptoms, and radiographical and microbiological findings.

Continuous data were presented as median and interquartile range (IQR1: 25th percentile, IQR3: 75th percentile). Categorical variables were compared using Fisher's exact test or chi-squared test when multiple groups were compared. Continuous variables were compared using Mann-Whitney test or Kruskal-Wallis test followed by Dunn's comparison. The Institutional Review Board (IRB) of CUIMC approved this study (#AAAT0009).

3 | RESULTS

The 18 patients included in this study had a median age of 55 (IQRs: 50, 61) years. Six of the patients were female and five were Black. Underlying etiologies of native kidney failure included immune complex-mediated glomerulonephritis ($n = 5$), ANCA-associated glomerulonephritis ($n = 1$), diabetic nephropathy ($n = 4$), hypertension ($n = 2$), smoking-related nodular glomerulosclerosis ($n = 1$), cystic kidney disease ($n = 2$), calcineurin inhibitor toxicity following liver transplantation ($n = 1$), congenital abnormalities ($n = 1$), and neurogenic bladder ($n = 1$). Six allografts were from living donors. All patients had comorbidities, including hypertension in 17 and diabetes mellitus in eight (Table 1).

Fifteen patients had developed COVID-19 pneumonia. Using the World Health Organization classification of COVID-19 severity,¹⁸ one patient was asymptomatic, two were mild, six moderate, four severe, and five critically severe, requiring intubation and mechanical ventilation (Table 1). Allograft biopsies were performed for AKI with proteinuria ($n = 4$), AKI alone ($n = 11$), isolated proteinuria ($n = 2$), and transplant nephrectomy after allograft failure ($n = 1$).

As demonstrated in Table 2, excluding the three patients who were dialysis-dependent prior to biopsy, the median baseline serum creatinine was 1.5 mg/dL (IQRs: 1.3, 2.2) and the median serum creatinine at biopsy was 2.4 (IQRs: 2.1, 2.8). Five patients had nephrotic range proteinuria. Although serial SARS-CoV-2 PCR by nasal swab was not performed for all patients, at least one patient had prolonged PCR positivity for over 6 months (patient #8).

Most kidney transplant recipients with COVID-19 were treated by lowering immunosuppression ($n = 13$; of which three were lowered following allograft biopsy) and/or initiation of therapy with corticosteroids ($n = 8$), tocilizumab ($n = 3$), remdesivir ($n = 3$), and/or bamlanivimab monoclonal antibody ($n = 2$) (Table 2).

TABLE 1 Patient demographics

Pt#	Age (yr)	Sex	Race	Cause of ESRD	Transplant source	Comorbidities	COVID-19 manifestations	WHO classification of COVID-19 severity	Biopsy indication
Biopsies within 1 month of positive PCR									
1	54	M	White	IgAN	Living	HTN, obesity	Asymptomatic	Asymptomatic	AKI
2	51	F	Hispanic	SLE	Deceased	HTN	Hypoxia respiratory failure requiring intubation	Critically severe	AKI, proteinuria
3	50	M	Hispanic	DM	Deceased	HTN, DM, obesity	Fever	Moderate	AKI
4	15	F	Hispanic	Decreased nephron mass	Deceased	HTN, obesity	Cough, fever, bilateral opacities on imaging	Moderate	AKI
5	71	M	White	Nodular GS (smoking related)	Deceased	HTN, smoking	Hypoxia	Severe	AKI, proteinuria
6	64	M	Hispanic	DM	Deceased	HTN, DM	Chest pain, fever, hypoxia, diffuse ground glass appearance on imaging	Severe	Proteinuria
7	55	F	Black	SLE	Living	HTN, former smoker	Abdominal pain, diarrhea, chills, hypoxia	Severe	AKI, proteinuria
8	54	F	Hispanic	PCKD	Deceased	HTN	Sore throat	Mild	AKI
9	22	M	Black	MN	Deceased	HTN	Cough, fever, bilateral infiltrates on imaging, requiring intubation	Critically severe	Nephrectomy for allograft failure
10	53	F	White	DM	Living	HTN, DM	Nausea, vomiting, loss of taste, cough	Moderate	AKI
11	66	M	White	PCKD	Deceased	HTN, DM	Cough, hypoxia, respiratory failure requiring intubation	Critically severe	AKI
Biopsies >1 month post-positive PCR									
12	61	M	Unknown	MN	Living	DM	Hypoxia	Moderate	AKI, proteinuria
13	61	M	Black	HTN	Deceased	HTN	Myalgias, cough, fever, hypoxia	Severe	AKI
14	40	F	Black	HTN	Deceased	HTN, DM	Diarrhea, cough, fever, hypoxic respiratory failure requiring intubation	Critically severe	AKI
15	58	M	Black	DM	Deceased	HTN, DM	Fatigue, loss of appetite	Mild	Proteinuria
16	45	M	Hispanic	Neurogenic bladder	Deceased	HTN	Cough, fever	Moderate	AKI
17	58	M	White	Pauci-immune crescentic GN	Living	HTN	Fatigue, cough, fever	Moderate	AKI
18	73	M	Hispanic	CNI toxicity	Living	HTN, DM, obesity	Cough, fever, hypoxia, respiratory failure requiring intubation	Critically severe	AKI

Abbreviations: AKI, acute kidney injury; CNI, calcineurin inhibitor; DM, diabetes mellitus; ESRD, end-stage renal disease; F, female; GN, glomerulonephritis; GS, glomerulosclerosis; HTN, hypertension; IgAN, IgA nephropathy; M, male; MN, membranous nephropathy; PCKD, polycystic kidney disease; PCR, polymerase chain reaction; Pt, patient; SLE, systemic lupus erythematosus; WHO, World Health Organization.

TABLE 2 Laboratory findings and follow-up information

Pt#	Baseline Scr (mg/dL)	Scr at biopsy (mg/dL)	UPCR (g/g)	Urine RBCs/hpf	Salb (g/dL)	Dialysis at biopsy	Duration of follow-up (days)	Post-COVID-19 therapy	Scr on follow-up (mg/dL)
Biopsies within 1 month of positive PCR									
1	1.7	2.6	2	60	4.5	No	363	Tocilizumab, IVIg, thymo, steroids, MMF reduced (after biopsy)	2.2
2	1.6	2.4	19.9	4	2.6	No	387	IVIg	Graft failure
3	1.1	1.7	<0.1	N/A	3.8	No	381	Thymo, steroids	1.4
4	0.5	2.1	0.31	272	4	No	182	Steroids, bamlanivimab (8 days before biopsy)	2.0
5	1.4	2.5	4.4	15	3.8	No	103	MMF held (after biopsy)	Graft failure
6	2	2.5	9.8	<5	3.2	No	32	Tacro and MMF reduced, bamlanivimab (31 days before biopsy)	Death with functioning graft
7	1.5	3.3	5.8	17	2.7	Yes	210	Steroids, MMF and prednisone held (after biopsy)	4.0
8	2.5	2.9	0.2	3	4.4	No	392	MMF reduced	1.2
9	Dialysis	Dialysis	N/A	N/A	3.4	Yes	432	Hydroxychloroquine, tocilizumab, pip-tazo, azithromycin	Graft failure before biopsy
10	1.2	1.8	0.2	1	4.1	No	377	MMF reduced	1.5
11	Dialysis	Dialysis	100 mg/dL	N/A	2.9	Yes	75	Steroids, MMF held, remdesivir (117 days before biopsy)	Graft failure before biopsy
Biopsies >1 month post-positive PCR									
12	2.6	7.3	9.0	3-5	3	No	151	MMF held	Graft failure a few days after biopsy
13	2.6	3.0	0.2	10	4.2	No	109	Steroids, ceftriaxone, azithromycin, azathioprine held, remdesivir (82 days before biopsy)	3.1
14	1.4	2.0	N/A	3	4.1	No	327	Tocilizumab, MMF held	2.6
15	2.3	2.3	2.5	13	4	No	71	Steroids, MMF held	2.3
16	1.5	2.2	0.2	1	4.3	No	373	MMF reduced	2.0
17	1.1	1.3	0.1	16	4.9	No	103	MMF held	1.24
18	Dialysis	Dialysis	100 mg/dL	11	2.2	Yes	21	Steroids, remdesivir (97 days before biopsy)	Graft failure before biopsy; patient expired 21 days after biopsy

Abbreviations: hpf, high power field; IVIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; N/A, not available; PCR, polymerase chain reaction; pip-tazo, piperacillin-tazobactam; Pt, patient; RBC, red blood cells; Salb, serum albumin; Scr, serum creatinine; tacro, tacrolimus; thymo, thymoglobulin; UPCR, urine protein to creatinine ratio.

TABLE 3 Immunologic characteristics

Pt#	Diagnosis	Time post-tx	DSA at transplant	IS	Inflammatory markers	CNI levels ^a	DSA at Bx	Prior acute rejection
Biopsies within 1 month of positive PCR								
1	TCMR, 2A	1 month	Pos	Tacro, MMF, pred	IL-6 7 pg/mL	11-16.5	Pos	No
2	AMR (g3, ptc3) + borderline TCMR	9 months	Pos	Tacro, MMF, pred	D-dimer >20 ug/mL; CRP >300 mg/L; ESR 75 mm/hr; LDH 625 U/L; IL-6 >315 pg/mL	5.4-6.3	Pos	1 month post-tx: mild AMR (g1, ptc1) + borderline TCMR
3	TCMR, 2B	6 months	Neg	Bela, MMF	N/A	N/A (Bela ^b)	Neg	No
4	TCMR, 2B	7 years	Neg	Tacro, pred	CRP 22mg/L; ESR 66 mm/hr; D-dimer 0.71ug/mL	<2.0-14	Pos	5 years post-tx: AMR (g0, ptc2, C4d3)
5	Proliferative GN with masked deposits +TCMR, 2A	40 days	Neg	Bela, MMF	IL-6 59 pg/mL; IL-2R 390 pg/mL; CRP 141 mg/L; D-dimer 4.57 ug/mL; LDH 312 U/L; ferritin 3394 ng/mL	N/A (Bela ^b)	Neg	No
6	Collapsing glomerulopathy	1.5 years	Neg	Tacro, MMF	N/A	5.8-6.0	Neg	13 months post-tx; mixed AMR (g2, ptc0, C4d1) + borderline TCMR
7	Recurrent LN with lupus podocytopathy	8 years	Neg	Tacro, MMF, pred	Ferritin wnl; LDH 469 U/L	2-11	Neg	No
8	ATI	53 days	Neg	Tacro, MMF	CRP wnl; ferritin 1677 ng/mL	8.5-12.5	Neg	No
9	Infarction	3 years	Neg	Tacro, pred	Ferritin 1630 ng/mL; ESR 79 mm/hr; CRP 196 mg/L; D-dimer 4.6 ug/mL; LDH 333 U/L	<2.0-3.1	Pos ^c	17 months post-tx mixed: AMR (ptc0-1, C4d3) + borderline TCMR
10	Transplant glomerulopathy	6 months	Pos	Tacro, MMF	N/A	6.8-12.6	Neg	No
11	ATI	5 months	Pos	Bela, MMF, pred	Ferritin 345 ng/mL	N/A (Bela ^b)	Neg	24 days post-tx Borderline TCMR
Biopsies >1 month post-positive PCR								
12	Collapsing glomerulopathy	1.5 years	Neg	Cyclosporine, MMF, pred	N/A	cyclosporine: 95-163	Neg	No
13	Moderate IFTA	1 year	Pos	Bela, IVIg, azathioprine	N/A	N/A (Bela ^b)	Neg	17 days post-tx TCMR, 2A
14	Severe IFTA	6 years	Pos	Bela, MMF, pred	IL-6 >315 pg/mL; ESR 85 mm/hr; Ferritin 4520 ng/mL; LDH 2078 U/L	N/A (Bela; held after COVID)	Neg	7 days post-tx AMR(C4d3, ATI)
15	Nodular diabetic GS, Moderate IFTA	13 months	Neg	Bela, tacro, MMF	N/A	N/A (Bela ^b)	Pos	No
16	Severe arteriosclerosis	3 months	Neg	Tacro, MMF	N/A	6.0-11.1	Pos	No
17	Mild IFTA	1 year	Neg	Tacro, MMF	N/A	8.7-13.9	Pos	No

(Continued)

TABLE 3 (Continued)

Pt#	Diagnosis	Time post-tx	DSA at transplant	IS	Inflammatory markers	CNI levels ^a	DSA at Bx	Prior acute rejection
18	ATI	4 months	Neg	Tacro, MMF	Ferritin 3296 ng/mL; CRP 226 mg/L; LDH 254 U/L	2.7 to 29	Neg	No

Abbreviations: AMR, antibody-mediated rejection; ATI, acute tubular injury; Bela, belatacept; Bx, biopsy; CNI, calcineurin inhibitor; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DSA, donor-specific antibody; ESR, erythrocyte sedimentation rate; g, glomerulitis; GN, glomerulonephritis; GS, glomerulosclerosis; IFTA, tubular atrophy and interstitial fibrosis; IS, immunosuppression; LDH, lactate dehydrogenase; LN, lupus nephritis; MMF, mycophenolate mofetil; N/A, not available; Neg, negative; Pos, positive; post-tx, posttransplant; pred, prednisone; Pt, patient; ptc, peritubular capillaritis; tacro, tacrolimus; TCMR, T cell-mediated rejection; Tx, transplant; wnl, within normal limits.

^aCalcineurin inhibitor (CNI) levels were assessed between the time of SARS-CoV-2 detection (by polymerase chain reaction) and allograft kidney biopsy, or 2 weeks before the biopsy if SARS-CoV-2 was detected less than 2 weeks before biopsy; tacrolimus levels measured in ng/mL; if the patient was maintained on cyclosporine, then this is mentioned and the levels are reported (ng/mL); if the patient was maintained on belatacept, then this is mentioned together with any missing doses.

^bDid not miss any belatacept infusion subsequent to detection of SARS-CoV-2.

^cConsidered positive since multiple prior values were positive for class-II DSA with high MFI (24,000 5 months prior to the current biopsy).

On analysis of histology results in the biopsies taken within 1 month of positive PCR ($n = 11$), the most common finding was acute rejection ($n = 5$, 45%, Table 3, Figure 1A), including three with moderate acute T cell-mediated rejection (TCMR: Banff grades 2B [$n = 2$] and 2A [$n = 1$]), one with antibody-mediated rejection (AMR) showing severe peritubular capillaritis and severe glomerulitis, and one with concurrent TCMR (Banff grade 2A) and proliferative glomerulonephritis with masked deposits that were apparent only after performing immunofluorescence on pronase-digested paraffin sections. In the latter biopsy, the arteritis was attributed to TCMR given the absence of deposits in vessel walls by conventional as well as pronase immunofluorescence. The biopsy performed at the time of COVID-19 showed marked increase in intraglomerular monocytes and the new development of arteritis (Figure 1B) compared to a previous biopsy performed several days before COVID-19, which had shown no arteritis but had masked deposits. Notably, this patient was found to have an IgG-kappa M-spike by serum electrophoresis with negative cryoglobulin testing.

For the patients with acute rejection, the time period from initial transplantation to biopsy ranged from 1 month to 7 years (Table 3). Two patients had preformed donor-specific antibodies (DSAs) and remained positive at the time of biopsy while one developed de novo DSAs 2 months prior to the diagnosis of COVID-19 and remained positive at the time of biopsy. Notably, none of these five patients had documented change in the immunosuppression regimen prior to the allograft biopsy (Table 2). Moreover, three of these five patients had no previous episodes of acute rejection and appeared adequately immunosuppressed (one with tacrolimus levels of 11–16.5 ng/mL within 2 weeks prior to biopsy and the other two were on regular belatacept infusion with no missed doses) (Table 3).

Two biopsies revealed podocytopathy, one with collapsing glomerulopathy (Figure 1C) and one with recurrent lupus nephritis with diffuse foot process effacement, consistent with lupus podocytopathy (Figure 1D). The patient with collapsing glomerulopathy (patient #6) was Hispanic and the patient with lupus podocytopathy (patient #7) was Black, whereas both of their donors were Black. Notably, the former had undetectable cytomegalovirus or Epstein-Barr virus DNA in the plasma within 3 months of biopsy and did not have critically elevated blood pressure (in the range of 130 mm Hg systolic), highly elevated tacrolimus levels, or concurrent acute rejection.

Two patients had ATI, one of them (patient #11) had critically severe COVID-19 while the other (patient #8) had mild COVID-19 (Table 1) without elevated tacrolimus levels (Table 3). One patient developed allograft infarction (patient #9). This patient had critically severe COVID-19 and his course was further complicated by long-standing anemia and underlying severe arteriosclerosis. Calcineurin inhibitor-mediated injury is unlikely in this case, as measured tacrolimus levels were low.

One biopsy demonstrated transplant glomerulopathy. This biopsy was performed only 6 months after transplantation as the first posttransplant biopsy. The cause was favored to be related to subacute TMA since DSAs were undetectable, C4d staining was

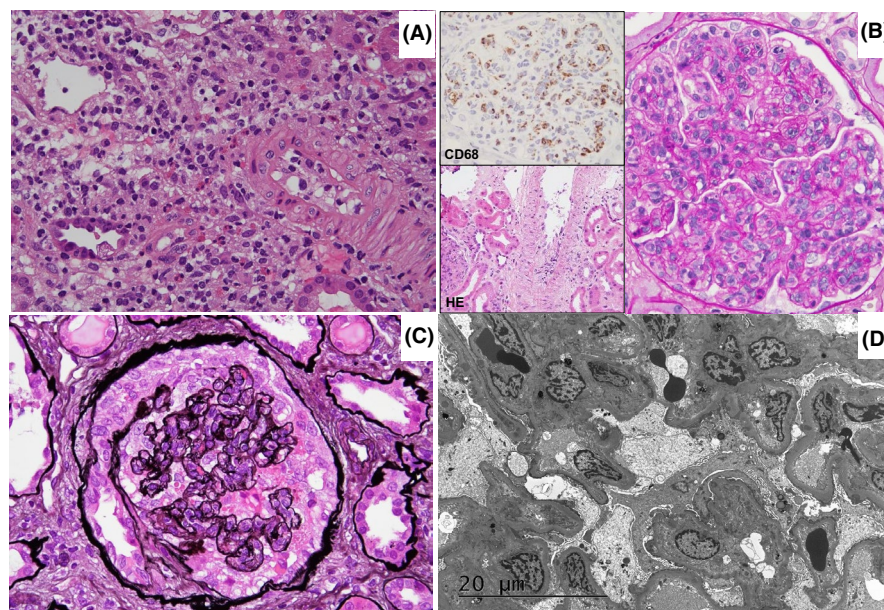


FIGURE 1 Kidney allograft biopsy findings in patients with COVID-19. (A) Light microscopy demonstrates diffuse disruptive mononuclear interstitial inflammation with scattered eosinophils and a focus of intimal arteritis in a small artery occluding >25% of the arterial luminal area in a patient with grade 2B acute T cell-mediated rejection (hematoxylin and eosin, original magnification $\times 400$). (B) Light microscopy showing a glomerulus with global endocapillary proliferation composed mainly of monocytes (upper inset; CD68 immunostaining) in a patient with masked deposits (periodic acid-Schiff, original magnification, $\times 400$). The biopsy also showed concurrent arteritis (lower inset, hematoxylin and eosin) that was not associated with immunofluorescence reactivity on pronase-digested sections. (C) Light microscopy demonstrates a lesion of collapsing glomerulopathy characterized by hyperplasia of glomerular epithelial cells and wrinkling of the glomerular basement membranes resulting in collapse of the glomerular tuft (Jones methenamine silver, original magnification, $\times 400$). (D) Diffuse foot process effacement out of proportion of the sparse subepithelial deposits in a patient with recurrent lupus nephritis with lupus podocytopathy (electron micrograph, original magnification, $\times 3000$)

negative in peritubular capillaries, tacrolimus levels were not highly elevated, and the patient did not have evidence for infection by hepatitis C virus.

The detailed histologic assessment of all allograft biopsies is presented in Table 4. To explore whether some of the observed pathologic findings might be specific for COVID-19, pathologic characteristics of our cohort of kidney allograft biopsies performed within 1 month of positive SARS-CoV-2 PCR test ($n = 11$) were paralleled first to two comparative groups: (a) 14 transplant patients with non-COVID-19 pneumonia within 1 month of kidney allograft histologic evaluation, and (b) 13 patients who underwent a native kidney biopsy within a month of COVID-19. Transplant patients with COVID-19 tended to have a higher incidence of acute rejection than transplant patients with non-COVID-19 pneumonia ($p = .06$), which reached statistical significance when the incidence of acute vascular rejection (TCMR grade 2A or above) was compared ($OR = 17$, $p = .03$) (Table S2). The incidence of other diagnoses did not reach statistical significance in this small sample. Compared to the other two groups, transplant patients with COVID-19 also had higher Banff scores for arteritis ($p = .005$), glomerulitis ($p = .005$), and peritubular capillaritis ($p = .02$) (Table S2).

In an attempt to confirm the above findings, the incidence of acute rejection and Banff scores associated with acute rejection were then compared with a historic cohort of all kidney allograft biopsies performed for allograft dysfunction that were reviewed at CUIMC in 2019 ($n = 538$, preceding the COVID-19 pandemic). Again,

allograft biopsies from transplant patients with COVID-19 had a significantly higher incidence of acute rejection ($OR = 3.5$, $p = .047$), especially acute vascular rejection ($OR = 11.6$, $p = .002$) and higher Banff scores for arteritis ($p < .001$) and, to a lesser extent, glomerulitis ($p = .02$) (Figure 2, Table S3).

In contrast to biopsies performed within 1 month of COVID-19, biopsies performed >1 month after positive SARS-CoV-2 PCR tended to show more chronic and nonspecific changes (Table 3). Only one additional case of a collapsing glomerulopathy (patient #12) was documented. In this case, the allograft kidney was obtained from a White donor and the biopsy demonstrated severe arteriolar hyalinosis and focal arteriolar thrombi consistent with vaso-occlusion. One biopsy revealed ATI (patient #18). This particular patient had severe COVID-19 and fluctuating tacrolimus levels. The remaining five patients had either nonspecific histologic findings ($n = 2$) or prominent chronic changes ($n = 3$), of which two had previous episodes of acute rejection that occurred prior to COVID-19 (Table 3).

When risk factors for acute rejection¹⁹ were compared between patients presenting more than 1 month from COVID-19 and those presenting within 1 month of diagnosis, no significant differences were noted in this small sample (Table S4).

Immunohistochemical staining for SARS-N-Capsid was negative in all 18 biopsies, *in situ* hybridization for SARS-CoV-2 RNA-encoding spike protein was negative in all of 17 biopsies, and no viral particles

TABLE 4 Pathologic characteristics

Pt#	Diagnosis	#glom	#GS	#SS	i	t	g	v	ptc	cg	ct	ci	cv	ah	ti	C4d	IF	EM	IHC/ISH
Biopsies within 1 month of positive PCR																			
1	TCMR, 2A	11	1	0	2	2	0	1	2	0	1	1	1	0	2	0	Minimal IgM-(donor-derived)	N/A	Neg/Neg
2	AMR +borderline TCMR	23	1	0	1	1	3	0	3	0	2	2	0	0	1	0	Neg	No VP	Neg/Neg
3	TCMR, 2B	7	0	0	3	3	1	2	0	0	1	1	1	0	3	0	Neg	N/A	Neg/ Neg
4	TCMR, 2B	13	2	0	3	3	0	2	2	0	1	2			0	0	Neg	No VP	Neg/N/A
5	Proliferative GN with masked deposits +TCMR, 2A	24	0	0	0	1	3	1	0	2	1	1	0	1	0	0	IgG 2+, kappa 2+, lambda 1+	~10% FPE; subendothelial lattice like organized substructure. No VP	Neg/Neg
6	Collapsing glomerulopathy	25	5	4	0	0	2	0	1	2	2	2	2	1	0	1	Neg	50% FPE, no VP	Neg/Neg
7	Recurrent LN with lupus podocytopathy	9	5	1	0	1	0	0	0	0	2	2	1	1	2	0	Sparse deposits along GC	~85% FPE, scattered subepithelial & intramembranous deposits, no VP	Neg/Neg
8	ATI	20	1	0	0	0	0	0	0	0	0	0	1	1	0	0	Neg	N/A	Neg/Neg
9	Infarction	NA	NA	NA	1	0	0	0	0	0	3	3	3	1	1	NA	N/A	N/A	Neg/Neg
10	Transplant glomerulopathy	24	3	0	0	0	1	0	0	2	1	1	1	0	0	0	N/A	N/A	Neg/Neg
11	ATI	11	0	0	0	1	0	0	2	0	1	0	1	1	1	0	Neg	N/A	Neg/Neg
Biopsies >1 month post-positive PCR																			
12	Collapsing glomerulopathy	19	10	3	0	1	0	0	0	0	3	3	0	3	3	1	Neg	N/A	Neg/Neg
13	Moderate IFTA	15	0	0	0	1	0	0	0	0	2	2	1	1	0	0	N/A	N/A	Neg/Neg
14	Severe IFTA	15	8	0	0	0	0	0	1	0	3	3	2	1	0	0	Neg	N/A	Neg/Neg
15	Nodular diabetic GS, Moderate IFTA	25	5	0	0	2	1	0	0	0	2	2	1	2	1	0	Neg	35-40% FPE, mesangial expansion, thickened GBMs. No VP	Neg/Neg
16	Severe arteriosclerosis	14	1	0	0	1	0	0	0	0	1	1	3	2	0	0	Neg	N/A	Neg/Neg
17	Mild IFTA	15	4	0	0	0	0	0	0	0	1	1	1	1	0	0	Neg	N/A	Neg/Neg
18	ATI	16	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Neg	N/A	Neg/Neg

Abbreviations: ah, arteriolar hyalinosis; AMR, antibody-mediated rejection; ATI, acute tubular injury; cg, chronic transplant glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, arterial fibrous intimal thickening; EM, electron microscopy; FPE, foot process effacement; g, glomerulitis; GBM, glomerular basement membrane; GC, glomerular capillaries; glom, glomeruli; GN, glomerulonephritis; GS, glomerular sclerosis; i, interstitial inflammation; IFTA, interstitial fibrosis and tubular atrophy; IgA, immunoglobulin A; IgG, immunoglobulin G; IHC, immunohistochemical staining for SARS-CoV-2; ISH, *in situ* hybridization for SARS-CoV-2; LN, lupus nephritis; N/A, not available; Neg, negative; PCR, polymerase chain reaction; Pt, patient; ptc, peritubular capillaritis; SS, segmental sclerosis; t, tubulitis; TCMR, T cell-mediated rejection; ti, total inflammation; v, intimal arteritis; VP, viral particles.

were seen in all six biopsies studied by electron microscopy (Table 4, Figure S1).

The follow-up period ranged from 1 to 14 months (median 210 days, IQRs: 103, 377). Two patients died (21 and 32 days after biopsy, Table 2). Death was attributed to gram negative sepsis in one patient (#18) and unknown causes in the other (patient #6) who was found unresponsive at home. Three patients were on dialysis at the time of the allograft biopsies and never recovered graft function, and three additional patients developed graft failure on follow-up (Table 2). Only two of the studied patients demonstrated marked improvement in kidney function approaching baseline levels.

4 | DISCUSSION

COVID-19 has been demonstrated to result in high morbidity and mortality in kidney transplant patients, with a mortality rate of 24% as compared to 1% in the general population.²⁰ While the mass deployment of vaccination against SARS-CoV-2 is encouraging, the transplant population remains vulnerable. Preliminary studies on the

efficacy of the mRNA vaccines on immunosuppressed patients suggest a weakened antibody response as compared to the general public.^{21,22} Until larger herd immunity is achieved or advances are made in dosing of the vaccine in transplanted patients, the dangers of COVID-19 and its effects on the kidney allograft are likely to persist.

AKI is reported to be a significant consequence of COVID-19 that affects both native and transplanted kidneys. Current theories regarding the cause of AKI in transplant patients with COVID-19 include direct viral invasion,^{8,9} possibly via the angiotensin-converting enzyme 2 (ACE2) receptor, or indirect allograft damage related in part to virus-induced systemic cytokine storm.¹² A number of series have been published regarding histologic findings in the native kidney of patients infected with SARS-CoV-2; however, a clear etiologic link is not always demonstrable. Even less information exists for the transplant population.

This series of kidney transplant recipients who underwent allograft biopsy after COVID-19 demonstrates a variety of causes of allograft dysfunction with a relatively poor outcome. Distinct disease entities were more frequent in biopsies obtained within 1 month of positive SARS-CoV-2 PCR compared to those obtained at later time points. During follow-up, two patients died and most

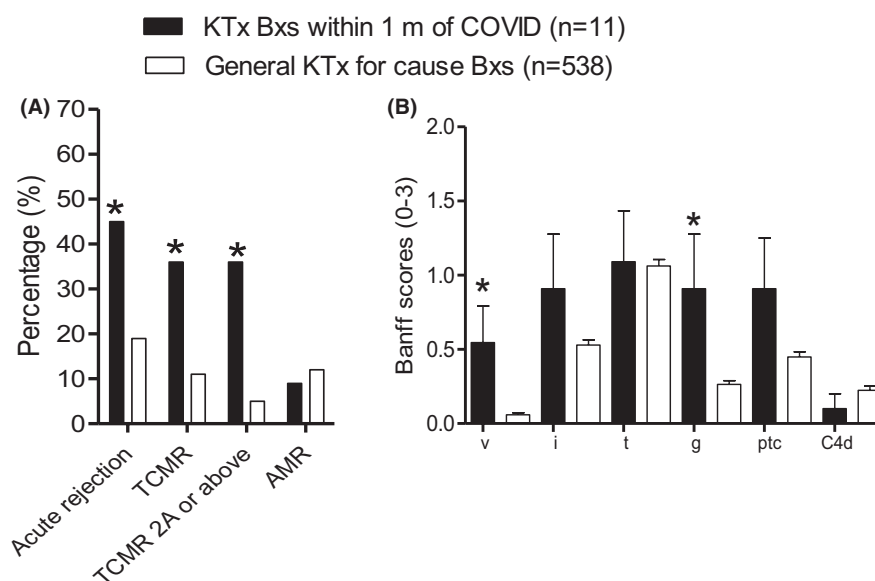


FIGURE 2 Comparison of pathologic findings in allograft biopsies within 1 month of COVID-19 and total “for-cause” kidney transplant biopsies. Histologic findings were compared between our cohort of allograft biopsies performed within 1 month of positive SARS-CoV-2 PCR ($n = 11$) and all “for-cause” allograft biopsies that were reviewed in 2019 at CUIMC ($n = 538$). (A) The diagnosis of acute rejection was more commonly seen in COVID-19 patients (OR = 3.5, $p = .047$). When the type of rejection was assessed, it became clear that the difference was attributed to acute T cell-mediated rejection (OR = 4.7, $p = .03$) and mainly to acute vascular rejection (OR = 11.6, $p = .002$) (B) When Banff scores for acute rejection were analyzed, it became apparent that the strongest difference was detected upon comparing arteritis scores ($p < .001$) followed by glomerulitis scores ($p = .02$). The latter need to be interpreted with caution since severe glomerulitis in one case is likely attributed to the presence of masked deposits. Of note, five general for cause allograft kidney biopsies had negative C4d staining in peritubular capillaries with histologic evidence of acute antibody-mediated tissue injury and evidence of current/recent evidence of antibody interaction with vascular endothelium but without available data on concurrent DSA. These five biopsies were classified as AMR. AMR, antibody-mediated rejection; Bxs, biopsies; KTx, kidney transplant; TCMR, T cell-mediated rejection (* $p < .05$; for more detailed analysis, please refer to Table S3)

patients displayed persistent renal dysfunction. In no case did we find evidence of direct viral infection of the allograft kidney either by immunohistochemistry, *in situ* hybridization, or electron microscopy.

The most common cause of allograft dysfunction in our series was acute rejection. Histologic evidence of acute rejection in the setting of COVID-19 has been previously documented.¹⁵ In two of the cases reported by Akilesh et al., an increase in DSA levels was noted around the time of allograft biopsy. In our series, three of the five patients who showed evidence of an acute rejection after COVID-19 had documented DSAs around the time of biopsy. This suggests the possibility that SARS-CoV-2 virus acts as a “second” hit in the setting of preexisting DSAs to trigger acute rejection. Although none of the five rejecting patients in our study had a documented decrease in their immunosuppression prior to the biopsy procedure, two had low tacrolimus levels between development of COVID-19 and allograft biopsy. Therefore, the possibility of acute rejection triggered by inadequate immunosuppression cannot be excluded in these two patients. Nevertheless, a high proportion of patients who had COVID-19 within 1 month of allograft biopsies developed acute rejection, especially acute vascular rejection (36%). The latter was more prevalent than a comparative group of transplant patients with other forms of pneumonia or a historic cohort of “for-cause” kidney allograft biopsies. Taken together, these findings suggest a high incidence of acute vascular rejection in transplant patients with COVID-19 that cannot be attributed entirely to reductions in immunosuppression.

Podocytopathies are also a predominant finding in the limited published literature regarding allograft dysfunction after COVID-19 (Table S5). There might be some differences between native and donor kidney disease with regard to the importance of APOL1 status. In the setting of COVID-19 in kidney transplant recipients, none of the two cases of collapsing glomerulopathy where the donor had been typed showed APOL1 high-risk genotypes.^{6,13} This is in contrast to COVID-19 in the native kidney where >90% of patients who developed collapsing glomerulopathy had APOL1 high-risk genotypes.^{4,13,23} In our series, podocytopathies were identified in three (17%) patients. While APOL1 genotypes of the donors were not tested in our cohort, we can assume that at least one of the two donors with collapsing glomerulopathy can be categorized as APOL1 low risk since he was White. However, it is worth noting that the kidney allograft biopsy in this particular patient also showed TMA, which is a known risk factor for collapsing glomerulopathy in the kidney allograft.²⁴

In our series, ATI was identified in three patients, of whom two had critically severe COVID-19 (management requiring intubation), a situation in which ATI is commonly observed.³

While this case series is the first to describe biopsy findings in kidney transplant recipients with COVID-19, it has several limitations. Given the descriptive nature of this study, it is difficult to prove a definitive pathogenetic link between SARS-CoV-2 infection and the biopsy findings. Our sample size is small, underscoring the need for additional multicenter studies. Finally, because all the allograft biopsies were performed for cause, our study cannot address potentially important subclinical histologic changes.

In conclusion, this series provides new insight into the factors underlying kidney injury in the kidney transplant population after SARS-CoV-2 infection. The close temporal associations with acute rejection implicate roles for heightened adaptive and innate immune responses.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author after obtaining an IRB approval. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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